

Potential Selection of Arbuscular Mycorrhizal Fungi (AMF) Indigenous Ultisols through the Production of Glomalin

Eddiwal¹, Amrizal Saidi², Ismon Lenin³, Eti Farda Husin² and Azwar Rasyidin²

¹Faculty of Agriculture, Islamic University of Western Sumatra; ²Faculty of Agriculture, University of Andalas,

³Institute for Agricultural Technology of West Sumatra.

Jl. Raya Halaban, Solok West Sumatra, Indonesia

e-mail: edwals.edu2@gmail.com

Received 23 May 2014 / accepted 30 August 2014

ABSTRACT

The arbuscular mycorrhizal fungi (AMF) with plants able to increase the capacity of plants to absorb nutrients and water from the soil. Recently, research was indicated that AMF hyphae containing glomalin as a glycoprotein that serves to unify the dispersed soil particles. The content of glomalin in soil is positively correlated with soil aggregate stability. The research potential of AMF species indigenous of Ultisol Darmasraya District of West Sumatra and glomalin production in experimental pots of sterile sand medium has been carried out. The purpose of this study was to determine the diversity of AMF species on Ultisol and to seeking indigenous AMF isolates that had the best glomalin production capability. AMF spores were isolated and identified from the rhizosphere soil of corn in Ultisol. AMF species that had been identified experimentally were tested in culture medium pot of sand and zeolite (w / w 1:1) using corn crops. The results found nine of the AMF species indigenous of Ultisol Darmasraya, namely *Acaulospora scrobiculata*, *Glomus etunicatum*, *Glomus luteum*, *Glomus mosseae*, *Glomus verruculosum*, *Glomus versiforme*, *Scutellospora gregaria*, *Scutellospora heterogama* and *Gigaspora* sp. AMF species that showed better colonization ability in corn was *G. luteum*, *G. verruculosum* and *G. versiforme*. All three species produced glomalin significantly higher than the other species, i.e. 1.29 mg g⁻¹; 1.17 mg g⁻¹; 1.15 mg g⁻¹, respectively.

Keywords: Glomalin, glycoprotein, indigenous, ultisol

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) can perform symbiosis with plants and are largely found in various ecosystems. The diversity of AMF in the soil may differ in environmental conditions from one another. AMF symbiosis with host plants can improve the absorption of nutrients and water for plants. Recent findings indicate that the role of the AMF to improve soil physical properties can be explained by increase in soil aggregation.

AMF activities has an important function in the ecosystem in the form of contributions to the improvement of soil structure that is highly correlated with soil aggregate stability and environmental quality (Wright and Upadhyaya 1998; Rillig and Mummey 2006; Treseder and Turner 2007; Borie *et al.* 2008). The role of AMF in soil aggregation is through AMF activity that produces a protein called glycoprotein

as “glomalin” (Wright *et al.* 2001). Glomalin is first discovered in 1996 (Wright and Upadhyaya 1996), in which this protein is abundant in the soil associated with the activity of AMF (Wright and Upadhyaya 1998). Rillig (2004) uses a new term to describe the soil protein which is called “glomalin-related soil protein (GRSP)” as the source of C (carbon) and N (nitrogen) soil and it is important in nutrient cycling and ecosystem (Treseder and Turner 2007; Hodge and Fitter 2010). Rillig *et al.* (2001) found that approximately 4 to 5% of the total soil C and N in Hawaii came from glomalin. Furthermore, Lovelock *et al.* (2004a) reported that levels of glomalin in the soil of La Selva at a depth of 0-10 cm was 3.94 mg g⁻¹, which contributed to soil C and N which were 3.2% and 5%, respectively.

Glomalin (GRSP) instead secretion of AMF hyphae, but more than 80% making up the walls of hyphae and spores of AMF (Driver *et al.* 2005). Glomalin form of adhesive (glue) is produced by the AMF for the transport of nutrients and water activity. Glomalin serves to protect the hyphae from drought and destruction attack by microbes, which after activity stalled hyphae (in weeks) apart along

the hyphae and glomalin fused with minerals (sand, dust and clay) and organic matter clump stable soil aggregates (Wright *et al.* 2001; Rillig 2004; Borie *et al.* 2008; Coraqueo *et al.* 2010). Wright and Nichols (2002) explained that the blob structures formed by binding soil glomalin is quite stable, resistant to water and wind erosion, porous enough to pass air and water. It can hold more water, preventing scale formation (crusting) in the surface layer of the soil and supports the development of plant roots.

Glomalin production by AMF hyphae during colonization of the plant root activity can differ between species. Wright *et al.* (1996) found that the amount of glomalin extracted per unit weight of hyphae differ between species *Gigaspora gigantea*, *Glomus intraradices* and *Glomus etunicatum*. Similar result was reported by Wright and Upadhyaya (1999), where the total production of glomalin was significantly different between the *Gi. rosea*, *G. caledonium* and *G. intraradices*. Furthermore Levelock *et al.* (2004b) observed between the result of different glomalin results of *Acaulospora morrowiae*, *Gi. rosea*, *G. etunicatum* and *G. intraradices*, where *A. morrowiae* produce the highest concentration of glomalin and *G. intraradices* the lowest.

Based on the above, the management of indigenous mycorrhizal fungi as biological resources of land, is a breakthrough technology for the development of sustainable agriculture. How large the potential diversity of AMF species indigenous to the Ultisol unknown. The research about the potential of AMF species to produce glomalin in corn is necessary needs to be done.

The purpose of this study was to determine the diversity of AMF species indigenous on Ultisol and seek isolates that have the best ability to produce glomalin.

MATERIALS AND METHODS

Exploration and Identification of AMF

Sampling site is on the District Darmasraya West Sumatra with soil order is Ultisol. Soil sampling was taken from two locations, sample taken from planting corn area at the Village of Pisang Berebus Sitiung and the Sungai Langkok Sitiung II. At each location 5 corn planting areas were taken for observation of root colonization and AMF spore, analysis of glomalin, aggregate stability, and soil chemical properties. Corn planting area measuring 0.5 to 1 hectare was used for random sampling. Soil sampling as much as 2.0 kg in the rhizosphere

of plants and their root samples were taken from each planting areas as many as 10 plants. Soil samples were taken at a depth of 5-20 cm, air dried for one week and put it in a plastic bag labeled and stored at a temperature of 15-20° C prior to analysis.

Research activities began with the observation of AMF colonization on the roots of corn plants. The roots were cleaned with tap water and cut into 2 cm. Roots were immersed in potassium hydroxide (KOH) 10% for 4 days. KOH was removed and rinsed with distilled water. HCl solution 1% was added and let it stand for one night. HCl then was discarded, 0.05% trypan blue dye was added. Colonization was measured by the gridline intersect method (Giovannetti and Mosse 1980). Soil samples indicated the presence of infection in the roots of corn followed AMF spore isolation and identified. Every single type of spore in one group was placed on a glass preparations (glass slides) in a mixture of 1:1 (v/v) of PVLG (Polyvinyl - Lacto - Glycerol) and Melzer's reagent for identification. Spores were identified by observing the type and morphology of spores by publication INVAM (<http://invam.caf.wvu.edu/fungi/taxonomy>) as well as a variety of sources such as Wilson *et al.* (1983), Morton (1988), Morton and Benny (1990) and Brundret (2004).

Tests on Indigenous AMF Isolates

AMF species that have been identified was breeding on sterile sand medium with corn plants for 6 weeks. AMF inoculation treatment as much as 30 spores in suspension introduced on the roots of corn plants were grown on a culture medium. AMF inoculant material from the culture medium showed colonization or spores contained in the media was used as a treatment in pot culture experiments.

Treatment of pot culture experiments were 9 species of AMF using a completely randomized design (CRD) with 3 replications. Treatment of mycorrhizal inoculation, namely; F0 = non-mycorrhizal (control); F1 = *A. scrobiculata*; F2 = *G. etunicatum*; F3 = *G. luteum*; F4 = *G. mosseae*; F5 = *G. verruculosum*; F6 = *G. versiforme*; F7 = *S. gregaria*; F8 = *S. heterogama* and F9 = *Gigaspora* sp.

Planting medium from a mixture of fine sand and zeolite (w / w 1:1) was sterilized. Extraction of sand was autoclaved by adding 100 mM sodium pyrophosphate pH 9.0 at 121° C for 1 hour. Na-pyrophosphate solution was poured and rinsed with distilled water. A mix sand and zeolite was repeated sterilization by autoclave for 15 minutes. Pots were filled with sterile medium and treated with 100 g of AMF inoculant at planting time.

Plants were maintained for 8 weeks sprinkled with distilled water and the provision of nutrient solution. Nutrient solutions were added every week, starting at the age of 2 weeks using nutrient solution according to Osaki *et al.* (1997). Standard nutrient solution compositions were : 30 mg $N L^{-1}$ (NH_4NO_3); 0.6-1.0 mg $P L^{-1}$ ($NaH_2PO_4 \cdot 2H_2O$); 30 mg $K L^{-1}$ (K_2SO_4 : $KCl = 1:1$); 50 mg $Ca L^{-1}$ ($CaCl_2 \cdot 2H_2O$); 20 mg $Mg L^{-1}$ ($MgSO_4 \cdot 7H_2O$); 2 mg $Fe L^{-1}$ ($FeSO_4 \cdot 7H_2O$); 0.5 mg $Mn L^{-1}$ ($MnSO_4 \cdot 4H_2O$); 0.5 mg $B L^{-1}$ (H_3BO_3); 0.2 mg $Zn L^{-1}$ ($ZnSO_4 \cdot 7H_2O$); 0.01 mg $Cu L^{-1}$ ($CuSO_4 \cdot 5H_2O$); 0.005 mg $Mo L^{-1}$ ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$).

Research Parameter and Analyzed Method

Parameters observed on soil sampling were normal stability index aggregates (Six *et al.* 2000), total glomalin (Wright *et al.* 2006; USDA_Glomalin Extraction), Diversity AMF (Dandan and Zhiwei, 2007) included spore density (SD), species richness (SR), Shannon-Wiener diversity index (H'), the isolation frequency (IF) and the relative abundance of spores (RA). Parameter observed on the planting medium pot experiment included a total glomalin and spore density (SD). Samples of plant roots were observed for the presence of AMF colonization.

Total glomalin was determined according to the USDA method (USDA_Bio-Rad Bradford Protein Assay). Briefly, 1 g of sample media / soil was added in 8 mL of 100 mM sodium pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$), pH 9.0 and autoclaved at 121°C for 1 hour. Samples were centrifuged 5000 rpm for 15 min, the supernatant was removed and repeated up to 3 times until the extracts was straw-colored or colorless. All supernatants were combined and measured the volume of extract then centrifuged 10,000 rpm for 10 minutes. Move as much as 1 ml of the supernatant was poured into a micro tube for measuring the content of glomalin.

Glomalin concentration was measured by a colorimetric Bradford protein assay (USDA method) using BSA (bovine serum albumin) as a standard. Measurements were conducted in 200 μl (micro liter) of PBS (Phosphate buffered saline), 10 μl of Bio - Rad dye reagent Coomassie Brilliant Blue R - 250 Staining Solution (produced by Bio - Rad Laboratories, Inc.) was added. Color reaction was read by microplate reader at a wavelength of 595 nm after 5 min. Optical density is measured and compared against a standard curve of known concentration (1.25 - 5.0 μl) of the BSA. In the PBS standard solution, the addition of 100 mM sodium pyrophosphate equivalent to sample volumes (200 μl PBS - sample volume) was added.

Statistical Analysis

The AMF community structure indigenous on Ultisol was determined by measuring the density of spores, species richness, relative abundance, frequency of isolation and the Shannon - Wiener diversity index. Spore density is a replication of AMF biomass. The relative abundance as a percentage of the number of spores of a species that demonstrates the ability of sporulation of AMF species. Frequency of isolation describe the distribution of AMF species in an ecosystem. All data were analyzed statistically using the Excel Analysis ToolPak and CoStat program version 8.0.

RESULTS AND DISCUSSION

The Diversity of Arbuscular Mycorrhizal Fungi on Ultisol of the Darmasraya District

The AMF species found in Ultisol Darmasraya District is as much as 9 species. Characteristics of the diversity of AMF in both locations can be seen in Table 1 and Figure 1. At the location of Pisang Berebus one species found in *Acaulospora*, 5 species of *Glomus*, 2 species of *Scutellospora* and 1 species of *Gigaspora*. In the Sungai Langkok found 7 species consists of 5 species of *Glomus*, 1 species of *Scutellospora* and 1 species of *Gigaspora*. Species of the genus *Glomus* was found predominantly in the Ultisol from both locations, while the other genera found little.

At the location of Pisang Berebus found as many as 2714 spores per 100 g of soil, which is the largest spores of *G. luteum* as many as 1009 spores and *G. versiforme* 999 spores which have relative abundance (RA) respectively 37.18% and 36.81%. In the Sungai Langkok found 367 spores per 100 g of soil with the highest number of spores of the species *G. versiforme* 134 spores (RA = 36.52%) and *G. luteum* 107 spores (RA = 29.16%). Fairly wide distribution of AMF species are at the location of Pisang Berebus (IF > 80%), but at the Sungai Langkok, AMF species have low prevalence. The diversity of AMF species at both locations showed that the location of Pisang Berebus there are species richness (SR) 7.8 and 4.8 in the Sungai Langkok. Value of the Shannon diversity index (H') is equal to 1.575 at the Pisang Berebus and 1,566 in the Sungai Langkok.

We found that the AMF *Glomus* is the type that has spread is quite extensive and dominant (IF > 80%) in the Ultisol Darmasraya, and more than 90% spores at both locations is derived from *Glomus*. It is almost as was also reported by Prihastuti (2007), where in the acid dry land in Central Lampung found





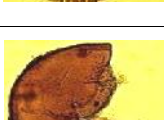

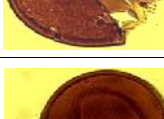
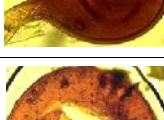
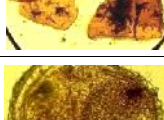
AFM morphology	Characteristics
	<i>Acaulospora scrobiculata</i> Spores pale yellow color, form spores globose / 300 μ m sieve rounded. Spore wall of three layers in which the outer layer is rather bright and pale yellow middle layer. Visible next to the separate components of the spore wall
	<i>Glomus etunicatum</i> Spores globose shaped reddish orange with a sieve size of 106 μ m at 53 μ m sieve. Spore wall of two layers, the outer arise turned a brownish red in Melzer's. Layer next to the colored bright yellow to arrange
	<i>Glomus luteum</i> Spores form globoses yellow orange sieve 106 μ m to 53 μ m. Four layers of the spore wall, outside light hyaline, pale yellow on the inside and a layer of rigid, no change of color in Melzer's
	<i>Glomus mosseae</i> Spores brownish orange, globus and slightly rounded shape. Sieve of 300 μ m to 106 μ m. There are 3 layers of the spore wall, the outer bright colors and turned into a pink reddish in Melzer's. The middle layer is rather light and separate the inner brownish yellow color.
	<i>Glomus verruculosum</i> Color yellow orange spore globose shape, sieve size of 300 μ m to 106 μ m. There are two layers of the spore wall, the outside such as light-colored peel and blend with the inner layer of yellow orange
	<i>Glomus versiforme</i> Spores brownish red, globose shaped with a size between 53 μ m and 106 μ m. Spore wall of two layers, the outer rather bright and not reacting in Melzer's. The inner layer of brownish yellow
	<i>Scutellospora gregaria</i> Spores brownish red color, globose shaped sieve size of 500 μ m at 300 μ m trapped. Spore wall of two layers, the outer rigid dark brown and light brown inner visible, reacting in Melzer's reddish
	<i>Scutellospora heterogama</i> Spores dark red slightly rounded shape / subglobose. Spore size sieve 300 μ m to 106 μ m. Outer spore wall light brown and reddish brown inner
	<i>Gigaspora</i> sp. Pale yellow spore color, shape rounded sieve size of 500 μ m at 300 μ m stuck. Spore wall seen two layers, the outer brown color and a reddish orange color inside. not react in Melzer's

Figure 1. The photograph spores of AMF indigenous on Ultisol in the District Darmasraya

8 species of AMF, including *G. mosseae*, *G. versiforme* and *Gi. margarita* is more dominant than others. In the hot dry valley areas in Southwestern China, Dandan and Zhiwei (2007) reported that there are six dominant species namely *G. clariodeum*, *G. clarum*, *G. fasciculatum*, *G. verruculosum*, *Glomus* sp 2 and *Gigaspora* sp1. They added that more than 80% of the total number of spores derived from *Glomus*. Lee and Eom (2009) was also reported that the AMF *Glomus* is dominant in soils from organic farms or in the conventional farms. The genus *Glomus* is often

dominant in agro-ecosystems (Bedini *et al.* 2007; Liu *et al.* 2009; Wu *et al.* 2010; Fokom *et al.* 2012).

Activity of AM fungi in the Pisang Berebus also better, which can be seen from the number of spores found 7 times more than in the Sungai Langkok. It is also supported by the content of total glomalin higher (3,604 mg g⁻¹) at the location of Pisang Berebus than the Sungai Langkok (1.008 mg g⁻¹). It can be understood that glomalin as building blocks in the walls of hyphae and spores (Driver *et al.* 2005), where an increase of activity AMF will generate high levels of glomalin. Differences of

glomalin content for both sites also appear to be associated with soil aggregate stability. Soil aggregate stability was positively correlated with the content of glomalin (Wright *et al.* 2000; Rillig *et al.* 2001; Rillig 2004; Wright and Nichols 2002).

Soil Aggregate Stability

Observations on the aggregate stability of both sites showed that the location of Pisang Berebus had an average value of NSI higher than the Sungai Langkok. The relationship between the content of glomalin to soil aggregate stability of the two locations can be seen in Figure 2.

Aggregate stability for the location of Pisang Berebus was better than Sungai Langkok with NSI values > 0.8 (Figure 2). The NSI values at the location of Sungai Langkok ranged from 0.6 - 0.74. Total glomalin at both location also showed the relationship with the NSI values and were positively correlated. Aumtung, (2010) have reported that the total glomalin was positively correlated with soil aggregate stability. He added that the cultivation of upland rice on dry land containing a total glomalin was higher than in lowland rice.

The existence of AMF in the soil plays an important role in soil aggregation through hyphae. Subowo (2010) has explained that the fungal hyphae is capable of uniting the soil aggregates bind to each other, so it is not easily damaged and resistant to physical stress or erosion. Hyphae and spores of the AMF is composed of 80% glomalin (Driver *et al.* 2005) that functions as an organic adhesive uniting stable soil aggregates (Wright *et al.* 2001). Hoorman *et al.* (2011) have explained that the three processes were simultaneously soil aggregation by AMF hyphae. At first, physically unite the hyphae

of soil particles. Secondly, fungi physically protect the clay and organic particles that make up the micro-aggregate. Finally, the plant root and fungal hyphae form glomalin that glues micro-aggregates and smaller macro-aggregates together to form larger macro-aggregates. Although bacterial effects on soil aggregate formation are found primarily on microaggregates but mycorrhizal effects are more evident on macroaggregates (Rillig and Mummey 2006).

The density of spores from both locations are also different (Table 1) which showed a positive correlation with the content of soil glomalin (Figure 3). The highest total glomalin found in the Pisang Berebus locations i.e. from 2.14 to 4.54 mg g^{-1} with spore density between 1824-4199 spores per 100 g soil. The Sungai Langkok location found a total glomalin $< 1.5 \text{ mg g}^{-1}$ and the number of spores between 155-637.

It is almost similar as was reported by Wu *et al.* (2010) where the density of AMF spores in the rhizosphere soil of wheat ranged from 350-1,380 per 50 g of soil. He added that the content of glomalin was negatively correlated with ratio C/N, but positively with the density of spores. Furthermore, Bai *et al.* (2009) reported a significant and positive relationship between the density of spores and glomalin. Thus, the evidence indicates that glomalin is contained in AMF spores and hyphae wall (Driver *et al.* 2005; Purine and Rillig 2007), so the greater spore production by AMF may indicate higher glomalin production. Fokom *et al.* (2013) has explained that the high positive correlation between total glomalin and soil water stable aggregate (WSA), carbon and total glomalin, organic matter and WSA as well as organic matter and total glomalin suggests

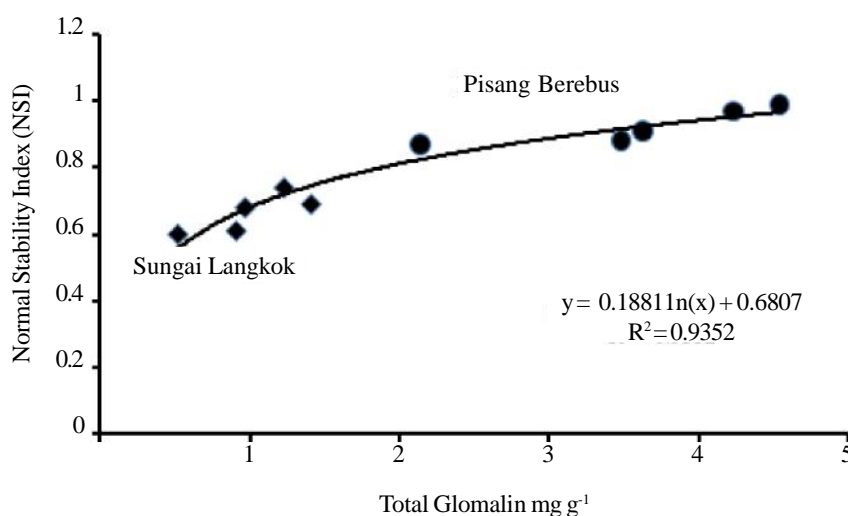


Figure 2. Relations between glomalin content with normal satibilitas index of soil aggregates on the Pisang Berebus and Sungai Langkok.

Table 1. Characteristics of the diversity of arbuscular mycorrhizal fungi.

No	Arbuscular mycorrhizal fungi	Pisang Berebus			Sungai Langkok		
		Spore Number	RA (%)	IF (%)	Spore Number	RA (%)	IF (%)
1	<i>A. scorbiculata</i>	35	1.29	80	0	0	0
2	<i>G. etunicatum</i>	172	6.34	80	42	11.44	80
3	<i>G. luteum</i>	1009	37.18	100	107	29.16	100
4	<i>G. mosseae</i>	159	5.86	100	36	9.81	80
5	<i>G. verruculosum</i>	183	6.74	80	32	8.72	40
6	<i>G. versiforme</i>	999	36.81	100	134	36.52	100
7	<i>S. gregaria</i>	79	2.91	100	12	3.3	40
8	<i>S. heterogama</i>	42	1.55	60	0	0	0
9	<i>Gigaspora sp.</i>	36	1.33	80	4	1.09	40
Total		2714	100		367	100	

that glomalin, C and N may contribute to the formation of WSA, and thereby promote the build up of soil structure. Guo *et al.* (2012) added that glomalin may be an appropriate index for evaluating soil fertility. Thus, that glomalin can be used as an indicator of soil quality (Fokom *et al.* 2012).

Based on the above results, it can be stated that the activities of the AMF can be explained from the measured total glomalin. The higher total glomalin illustrated the number of hyphae and spores produced by AMF were too much. It can be understood that glomalin are the building blocks of hyphae and spores wall. This component is produced during symbiosis activities with plants, as described by Wright and Upadhyaya (1996) and Driver *et al.* (2005).

The Content of Glomalin in Cultur Media

Each species of AMF will have the ability to grow and develop differently during activity symbiosis with the host plant. One way to determine the activity of AMF symbiosis by measuring the levels of glomalin produced.

The results test of 9 species showed significantly different glomalin content, especially *G. verruculosum*, *G. versiforme* and *G. luteum* (Table 2). The highest levels of glomalin was *G. verruculosum* (1.29 mg g⁻¹), followed by *G. versiforme* was 1.17 mg g⁻¹ and *G. luteum* was 1.15 mg g⁻¹. *Glomus mosseae* (0.65 mg g⁻¹) showed the same effect with *S. gregaria* and *S. heterogama*. Total glomalin extracted ranged of 0.18 to 1.29 mg g⁻¹ soil, the lowest in the range reported by Wright and Upadhyaya (1998). Wright and Upadhyaya (1998) reported glomalin concentrations ranged from 1 to

21 mg g⁻¹ soil in thirty-seven soil of five geographical locations. On the other hand, the concentration of glomalin in pot trial study by Antibus *et al.* (2006) ranged from 1-5.5 mg g⁻¹ soil. This was also confirmed by Lovelock *et al.* (2004b) that the glomalin concentration was low for pot cultivation.

Interestingly, it turns out the concentration of glomalin or proteins were also measured on the treatment without AMF inoculation. The concentration of glomalin in the planting medium was the lowest in the treatment with no inoculation (0.0367 mg g⁻¹), which showed no significant difference by treatment with *A. scorbiculata* inoculation. However, both of these treatments were statistically significantly different with other treatments. According to Rosier *et al.* (2008), a protein produced by organisms other than the AMF in the rhizosphere can be detected. He also found

Table 2. Levels of glomalin production (mg g⁻¹) in the growing media.

No	Inoculation Treatment of FMA	Average glomalin production (mg.g ⁻¹)
1	<i>G. verruculosum</i>	1.2900 a
2	<i>G. versiforme</i>	1.1667 a
3	<i>G. luteum</i>	1.1533 a
4	<i>G. mosseae</i>	0.6467 b
5	<i>S. gregaria</i>	0.4833 bc
6	<i>S. heterogama</i>	0.4633 bcd
7	<i>Gigaspora sp.</i>	0.4400 cd
8	<i>G. etunicatum</i>	0.2733 de
9	<i>A. Scrobiculata</i>	0.1767 ef
10	Non-mycorrhizal	0.0367 f

The numbers followed by the same letter are not significantly different ($P = 0.05$) according to LSD test of one-way ANOVA.

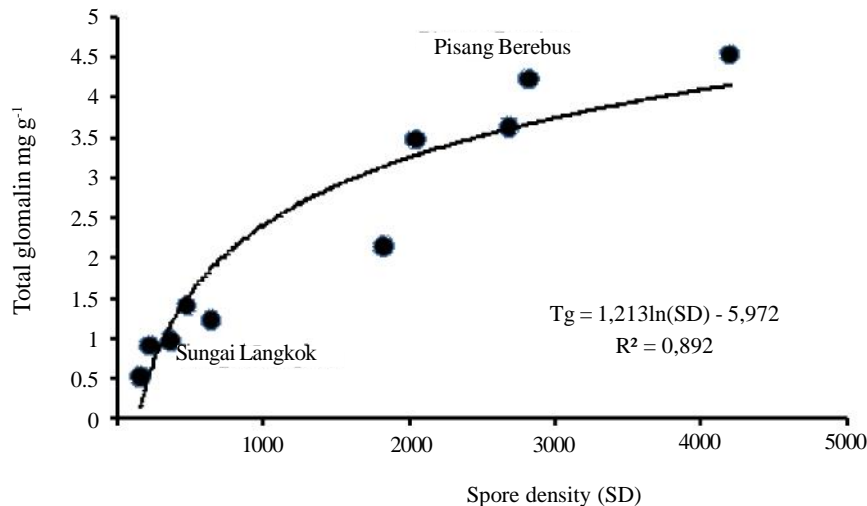


Figure 3. Relations between spore density with glomalin content of the soil in The Pisang Berebus and Sungai Langkok

measuring by Bradford protein assay contained glomalin on treatment without AMF inoculation were not significantly different with the inoculation of *G. intraradices* or *Entrophospora colombiana*.

Glomus tends to produce higher concentrations of glomalin, where the results are more varied with different AMF species. Therefore, differences in glomalin production by nine of indigenous AMF species were statistically different. The same thing Nichols and Wright (2004) also reported that glomalin concentrations varied between five species of AMF, namely: *G. etunicatum*, *G. viscosum*, *G. caledonium*, *Gi. rosea* and *Gi. gigantea*. In fact, they reported differences between isolates of the same species. Lovelock *et al.* (2004b) reported that glomalin production varies significantly across species of AMF in pot culture with maize as a host plant. They found that *A. morrowiae* produced glomalin higher than *Gi. rosea*, *G. etunicatum* and *G. intraradices*, and glomalin production by *G. intraradices* significantly lower than *Gi. rosea* and *G. etunicatum*. The differences in glomalin production may be due to differences in the activity of fungi (Bedini *et al.* 2007), and environmental stress conditions can affect the production of glomalin (Hammer and Rillig 2011).

CONCLUSIONS

This study is a pioneer to determine the mycorrhizal diversity in Ultisol and glomalin production of corn from farmland in the District Darmasraya West Sumatra. Our results indicated that the density of spores and AMF community were varied in the soil. We found there were nine of

indigenous AMF species, where *Glomus* was relatively dominant in the rhizosphere soil of corn. In pot culture test, the results showed that the species of *G. verruculosum*, *G. versiforme* and *G. luteum* produces glomalin was significantly higher than the other species. Total glomalin of the three species were 1.29; 1.17 and 1.15 mg g⁻¹ of soil respectively.

ACKNOWLEDGEMENTS

The authors would like to thank the Agency for Agricultural Research and Development of the Republic of Indonesia, through a partnership of national agricultural research and development (KKP3N) for the financial support to this research, the project contract No. 725/LB.620/I.1/ 2/2013.

REFERENCES

- Antibus RK, C Lauber, RL Sinsabaugh and DR Zak. 2006. Responses of Bradford-reactive soil protein to experimental nitrogen addition in three forest communities in northern lower Michigan. *Plant Soil* 288: 173-187.
- Aumtung S, P Sirinikorn, P Susingsa and N Maungjai. 2010. Glomalin-related soil protein influence on soil aggregate stability in soil of cultivated areas and secondary forests from Northern Thailand. Papersth World Congress of Soil Science, Soil solutions for a Changing World. 1 – 6 August 2010, Brisbane, Australia.
- Bai C, X He, H Tang, B Shan and L Zhao. 2009. Spatial distribution of arbuscular mycorrhizal fungi, glomalin and soil enzymes under the canopy of *Astragalus adsurgens* Pall. in the Mu Us sandland, China. *Soil Biol Biochem* 41: 941-947.

- Bedini S, L Avio, E Argese and M Giovannetti. 2007. Effects of long-term land use on arbuscular mycorrhizal fungi and glomalin-related soil protein. *Agric Ecosyst Environ* 120: 463-466.
- Borie F, R Rubio and A Morales. 2008. Arbuscular mycorrhizal fungi and soil aggregation. *R C Suelo Nutr Veg* 8: 9-18.
- Brundrett M. 2004. Diversity and classification of mycorrhizal associations. *Biol Rev* 79: 473-495.
- Curaqueo, GE Acevedo, P Cornejo, A Sequel, R Rubio and F Borie. 2010. Tillage effect on soil organic matter mycorrhizal hyphae and aggregates in a Mediterranean agroecosystem. *RC Suelo Nutr Veg* 10: 12-21.
- Dandan Z and Zhiwei Z. 2007. Biodiversity of arbuscular mycorrhizal fungi in the hot-dry valley of the Jinsha River, Southwest China. *Appl Soil Ecol* 37: 118-128.
- Driver JD, WE Holben and MC Rillig. 2005. Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi. *Soil Biol Biochem* 37: 101-106.
- Fokom R, S Adamou, MC Teugwa, AD Begoude Boyogueno, WL Nana, MEL Ngonkeu, NS Tchameni, D Nwaga, G Tsala Ndzomo and PH Amvam Zollo. 2012. Glomalin related soil protein, carbon, nitrogen and soil aggregate stability as affected by land use variation in the humid forest zone of south Cameroon. *Soil Till Res* 120: 69-75.
- Fokom R, MC Teugwa, WL Nana, MEL Ngonkeu, NS Tchameni, D Nwaga, MC Rilig and PH Amvam Zollo. 2013. Glomalin, carbon, nitrogen and soil aggregate stability as affected by land use changes in the humid forest zone in South Cameroon. *Appl Ecol Environ Research* 11: 581-592.
- Giovannetti M and B Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol* 84: 489-500.
- Guo H, X He and Y Li. 2012. Spatial distribution of arbuscular mycorrhiza and glomalin in the rhizosphere of *Caragana korshinskii* Kom. in the Otindag sandy land, China. *Afr J Microbiol Res* 6: 5745-5753.
- Hammer EC and MC Rillig. 2011. The influence of different stresses on glomalin levels in an arbuscular mycorrhizal fungus-salinity in creases glomalin content. *Plos ONE* 6: 1-5.
- Hodge A and AH Fitter. 2010. Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. *PNAS* 107: 13754-13759.
- Hoorman JJ, JC de Moraes (Juca) Sa and R Reader. 2011. The Biology of Soil Compaction. American Society of Agronomy. *Crops Soils Magazine* (July-August 2011): 4-10.
- INVAM__ Species Description from Reference cultures [Online]. Available at <http://invam.caf.edu/fungi/taxonomy>.
- Lee JE and AH Eom. 2009. Effect of Organic Farming on Spore Diversity of Arbuscular Mycorrhizal Fungi and Glomalin in Soil. *Mycobiology* 37: 272-276.
- Liu Y, L He, L An, T Helgason and H Feng. 2009. Arbuscular mycorrhizal dynamics in a chronosequence of *Caragana korshinskii* plantations. *FEMS Microbiol Ecol* 67: 81-92.
- Lovelock CE, SF Wright, DA Clark and RW Ruess. 2004a. Soil stocks of glomalin produced by arbuscular mycorrhizal fungi across a tropical rain forest landscape. *J Ecol* 92: 278-287.
- Lovelock CE, SF Wright and KA Nichols. 2004b. Using glomalin as an indicator for arbuscular mycorrhizal hyphal growth: an example from a tropical rainforest soil. *Soil Biol Biochem* 36: 1009-1012.
- Morton JB. 1988. Taxonomy of V A mycorrhizal fungi : Classification, nomenclature, and identification. *Mycotaxon* 32: 267-324.
- Morton JB and GL Benny. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes) : A new order, glomales, two new sub orders glomineae and Gidasporineae, and two new families *Acaulospora cease* and gidasporaceae with an emendation of Glomaceae. *Mycotaxon*. XXXVII : 471-491.
- Nichols KA and SF Wright. 2004. Contributions of fungi to soil organic matter in agroecosystems. In: F Magdoff and RR Weil (eds), *Soil Organic Matter in Sustainable Agriculture*. CRC Press, Florida, pp. 179-198
- Osaki M, T Watanabe and T Tadano. 1997. Beneficial effect of aluminum on growth of plants adapted to low pH soils. *Soil Sci Plant Nutr* 43: 551-563.
- Prihastuti. 2007. Isolasi dan karakterisasi mikoriza vesikular arbuskular di lahan kering masam Lampung Tengah. *Penelitian Hayati*. 12: 99-106 (in Indonesian).
- Purin S and MC Rillig. 2007. The arbuscular mycorrhizal fungal protein glomalin: Limitations, progress, and a new hypothesis for its function. *Pedobiologia*. 51: 123-130.
- Rillig MC, SF Wright, KA Nichols, WF Schmidt and MS Torn. 2001. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical rain forest. *Plant and Soil*. 233: 167-177.
- Rillig MC. 2004. Arbuscular mycorrhizae, glomalin and soil aggregation. *Can J Soil Sci* 84: 355-363.
- Rillig MC and DL Mummey. 2006. Mycorrhizas and soil structure. *New Phytol*. 171: 41-53.
- Rosier CL, SJ Piotrowski, AT Hoyer and MC Rillig. 2008. Intraradical protein and glomalin as a tool for quantifying arbuscular mycorrhizal root colonization. *Pedobiologia* 52: 41-50.
- Six J, ET Elliott and K Paustian. 2000. Soil structure and soil organic matter : II. A normalized stability index and the effect of mineralogy. *Soil Sci Soc Am J* 64: 1042-1049.
- Subowo G. 2010. Strategi efisiensi penggunaan bahan organik untuk kesuburan dan produktivitas tanah melalui pemberdayaan sumberdaya hayati tanah. *J Sumberdaya Lahan* 4: 13-25. (in Indonesian).
- Treseder KK and KM Turner. 2007. Glomalin and ecosystem. *Soil Sci Soc Am J* 71: 1257-1266.

- USDA BIO-RAD BRADFORD PROTEIN ASSAY - USDA Bio-Rad Bradford Total Protein Assay with Sodium Pyrophosphate Modification. Authors (Bradford 1976; Nichols and Wright 2004 and Wright *et al.* 2006) [Online]. Available at www.ars.usda.gov/.../Bradford%20Total%20Protein%20Assay.pdf
- USDA GLOMALIN EXTRACTION - USDA Glomalin Extraction with Sodium Pyrophosphate. Authors (Rillig 2004; Rosier *et al.* 2007; and Rillig 2003; Wright *et al.* 1996; Wright and Jawson 2001; Wright Nichols and Schmidt 2006; Wright and Upadhyaya 1996; and Wright and Upadhyaya 1998) [Online]. Available at www.ars.usda.gov/.../ad_hoc/54450000Glomalin/Glomalin%20Extraction.pdf.
- Wilson JM, MJ Trinick and CA Parker. 1983. The identification of vesicular arbuscular mycorrhizal fungi using immunofluorescence. *Soil Biol Biochem* 15: 439-445.
- Wright SF and A Upadhyaya. 1996. Extraction of an abundant an unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Sci* 161: 575-586.
- Wright SF, M Franke-Snyder, JB Morton and A Upadhyaya. 1996. Time course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. *Plant Soil* 181: 193-203.
- Wright SF and A Upadhyaya. 1998. A survey of soil for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil* 98: 97-107.
- Wright SF and A Upadhyaya. 1999. Quantification of arbuscular mycorrhizal activity by the glomalin concentration on hyphae. *Mycorrhiza*. 8: 283-285.
- Wright SF, MC Rillig and KA Nichols. 2000. Glomalin : A soil protein important in carbon sequestration. Proceedings of American Chemical. USDA-ARS-SMSL. Beltsville. [Online] Available at web.anal.gov/PCS/acsfuel/preprint archive/Files.
- Wright SF, KA Nichols, L Jawson, L McKenna and A Almendras. 2001. Glomalin-A manageable soil glue. Soil Science Society of America Special publication Book Chapter. 21 October 2001. ARS.USDA.gov.
- Wright SF and KA Nichols. 2002. Glomalin: Hiding place for a third of the world's stored soil carbon. *Agricultural Research Magazine*. September 2002 Volume 50 No. 9. [Online] Available at www.ars.usda.gov/is/AR/archive/sep02/soil0902.htm?pf=1
- Wright SF, KA Nichols and WF Schmidt. 2006. Comparison of efficacy of three extractants to solubilize glomalin on hyphae an in soil. *Chemosphere* 64: 1219 – 1224. www.elsevier.com/locate/chemosphere.
- Wu F, M Dong, Y Liu, X Ma, L An, J Peter, W Young and H Feng. 2010. Effects of long-term fertilization on AM fungal community structure and Glomalin-related soil protein in the Loess Plateau of China. *Plant and Soil @ Springer Science+Business Media B.V.* 2010: 10.1007/s11104-010-0688-4. Published online: 29 December 2010.